

simulations (~1 μ s each) were used to compare the open-state of the three human isoforms, obtaining a structural characterization particularly focused on the localization and mobility of N-termini (3) and its interaction with the β -barrel. In addition, each isoform was simulated in presence of KCl 0.5 M to investigate ions diffusion events, either in absence and presence of a 10 mV external electric field, in order to compare the selectivity, permeability ratio and ion currents.

The specific hydrophobic interactions and hydrogen bonds between N-terminal fragments and the barrel were found to be related to the intrinsic breathing motions of the latter. The overall shape of channels can be described as an ellipse, whose axes fluctuations are markedly anticorrelated in hVDAC1 and hVDAC3 while, essentially, no correlation was observed in hVDAC2.

Free-energy profiles of chloride and potassium ions reflect the anion selectivity of the isoforms with no significant differences in the average translocation time in both directions. However, a comprehensive explanation of differences between chloride and potassium was obtained with the cluster analysis that showed a preferential localization of the former in the middle of the pore while the latter 'segregated' at the periphery. The relative number of clusters in the three human isoforms of VDAC agrees with their relative selectivity. The position of the clusters matches with the charged residues distribution around the lumen.

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3846-Pos Board B574

Markov Chain Monte Carlo Model Analysis of Cardiac Mitochondrial VDAC1 Kinetics

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Metabolites, nucleotides, and ions are transported across the outer membrane of mitochondria (OMM) via the voltage-dependent anion channel, VDAC. Besides its role as the gatekeeper that regulates the metabolic and energetic functions of mitochondria, VDAC has also been implicated in apoptosis. In neurodegenerative and cardiovascular diseases, the most abundant isoform, VDAC1, has been shown to undergo post-translational modifications (PTMs). However, the impact of PTMs on VDAC1 characteristics and subsequent mitochondrial dysfunction are not well established. We developed a computational model that accurately described the effects of PTMs on VDAC1 gating. Channel activity was obtained from recombinant or purified cardiac VDAC1 incorporated into lipid bilayers. Recordings were acquired in the presence of a nitric oxide donor, PAPA NONOate (PPN), and from a VDAC1 phosphomimetic, S137E. For analysis of VDAC1 activity, we used Bayesian statistics combined with Markov Chain Monte Carlo (MCMC) sampling which provided an excellent method for identifying kinetic models and associated parameters. Unlike the maximum likelihood estimator, the MCMC method returned the probability distribution of the rate constants from which the model fitness and identifiability were reliably obtained. The gating of wild-type, S137E mutant, and VDAC1 exposed to PPN (25 and 100 μ M) could be described by a single kinetic model having five states with three distinct conducting states. Additionally, the model revealed transition kinetics that were not previously identified. Thus, our MCMC-based model allows for the prediction of VDAC1 activity under various PTM conditions. Our next stage is to integrate this kinetic model with a computational model of mitochondria to develop a comprehensive model that encompasses the changes in ionic flow across the OMM to more accurately predict mitochondrial dysfunction under pathophysiological conditions.

3847-Pos Board B575

A Lysosomal ATP-Sensitive Sodium Channel and its Regulation by Metabolism

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Both plasma and organelle membranes are known to have ionic permeability, but the molecular basis for the organelle ion conductances and their regulations are poorly understood. Using whole organelle current- and voltage-clamp recording from enlarged endosomes and lysosomes, we characterized the endolysosomal ion conductances. The organelles are highly permeable to H⁺ and their relative Na⁺ permeability (P_{Na}/P_K) can change drastically up to

28-fold under various conditions. Voltage-clamp recordings revealed ion channels permeable to K⁺, H⁺, Na⁺ and/or Cl⁻. In endolysosomes from macrophages, cardiac myocytes and neurons, we found an ATP-sensitive Na⁺ channel (lysoNaATP). The channel pore is formed by two-pore channel proteins (TPCs), proteins with similarity to the 24 transmembrane-spanning (4x6TM) plasma membrane Na⁺ and Ca²⁺ channels but with only half the size (2x6TM). The ATP sensitivity of the channel requires mTORC1. When extracellular nutrients (amino acids in particular) are present, mTOR translocates onto lysosomal membranes where it closes the TPC channel in a kinase- and ATP-dependent manner. Using knockout mice lacking TPCs, we found that the channel controls lysosomal pH stability, couples cell's energy and metabolic status to lysosomal function, and is required for animal's physical endurance during food shortage.

3848-Pos Board B576

A Thylakoid-Located Two-Pore K⁺ Channel Controls Photosynthetic Light Utilization in Plants

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The size of the light-induced proton motive force (pmf) across the thylakoid membrane of chloroplasts is regulated in response to environmental stimuli. Here, we describe a component of the thylakoid membrane, the two-pore potassium (K⁺) channel TPK3, which modulates the composition of the pmf through ion counterbalancing. Recombinant TPK3 exhibited potassium-selective channel activity sensitive to Ca²⁺ and H⁺. In Arabidopsis plants, the channel is found in the thylakoid stromal lamellae. Arabidopsis plants silenced for the TPK3 gene display reduced growth and altered thylakoid membrane organization. This phenotype reflects an impaired capacity to generate a normal pmf, resulting in reduced CO₂ assimilation and deficient non-photochemical dissipation of excess absorbed light. Thus, the TPK3 channel manages the pmf necessary to convert photochemical energy into physiological functions.

3849-Pos Board B577

Purified Functional Human Connexin 26 Hemichannels Expressed in E. Coli

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Gap junction channels communicate the cytoplasm of adjacent cells, and are formed by head-to-head association of two hemichannels, one from each of the adjacent cells. Gap-junction channels and hemichannels mediate electrical and chemical communication between cells due to their permeability to ions and hydrophilic molecules with molecular weights of up to 1,000 Da. Mutations of Cx26 are the most frequent cause of genetic deafness, and it is therefore important to understand the structure-function relationship of wild-type and deafness-associated mutants. Currently the preferred system for connexin overexpression is the insect cell/baculovirus system. This system has many drawbacks, including complexity and cost, which severely limit high-throughput screening of mutants. Here, we present an E. coli-based expression system for Cx26 that yields functional protein. The functional and biochemical properties of Cx26 hemichannels purified from insect cells and E. coli were indistinguishable. We also show that a poly-His tag fused to the C-terminal end affects hemichannel gating. We conclude that the E. coli-based expression system is suitable to produce milligram amounts of purified and functional Cx26 hemichannels. Supported by National Institutes of Health grants R01GM79629 and 3R01GM079629-03S1, Fondecyt #1120214, and Anillo ATC1104.

3850-Pos Board B578

Phospholamban is a Cation Selective Ion Channel

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Phospholamban (PLN) is a small integral membrane protein, which is involved in the contractility of cardiac muscle by regulating intracellular calcium concentration of cardiac myocytes. PLN binds and inhibits in a yet unknown way the sarco/endoplasmic reticulum Ca²⁺-ATPase. When reconstituted in planar lipid bilayers PLN exhibits ion channel activity with a main